

SEQUENCE LISTING

<110> Eisenberg, Stephen P.
 Case, Casey C.
 Cox III, George N.
 Jamieson, Andrew
 Rebar, Edward J.
 Sangamo Biosciences, Inc.

<120> Selection of Sites for Targeting by Zinc Finger
 Proteins and Methods of Designing Zinc Finger Proteins
 to Bind to Preselected Sites

<130> 019496-001800US

<140> US 09/229,007
 <141> 1999-01-12

<160> 97

<170> PatentIn Ver. 2.1

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 characterizing the C-2H-2 class of zinc finger
 proteins (ZFP)

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 Glu Leu Thr Arg His Ile Arg Ile His Thr Gly Gln Lys Pro Phe Gln
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 Cys Arg Ile Cys Met Arg Asn Phe Ser Arg Ser Asp His Leu Thr Thr
 35 40 45

 His Ile Arg Thr His Thr Gly Glu Lys Pro Phe Ala Cys Asp Ile Cys
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 Gly Arg Lys Phe Ala Arg Ser Asp Glu Arg Lys Arg His Thr Lys Ile
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 His Leu Arg Gln Lys
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 Val Tyr Gly Lys Thr Ser His Leu Arg Ala His Leu Arg Trp His Thr
 20 25 30

 Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly Lys Arg Phe
 35 40 45

Thr Arg Ser Asp Glu Leu Gln Arg His Lys Arg Thr His Thr Gly Glu
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Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met Arg Ser Asp
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Met Glu Lys Leu Arg Asn Gly Ser Gly Asp Pro Gly Lys Lys Lys Gln
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His Ala Cys Pro Glu Cys Gly Lys Ser Phe Ser Lys Ser Ser His Leu
20 25 30

Arg Ala His Gln Arg Thr His Thr Gly Glu Arg Pro Tyr Lys Cys Pro
35 40 45

Glu Cys Gly Lys Ser Phe Ser Arg Ser Asp Glu Leu Gln Arg His Gln
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Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
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Asn Lys

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gnngngggnnn nngngggngn gg

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 motif searched by protocol 1

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<223> Description of Artificial Sequence:target site DNA
      motif searched by protocol 1
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<223> Description of Artificial Sequence:target site DNA
      motif searched by protocol 1
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 motif searched by protocol 1

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<221> modified_base

<222> (1)..(19)

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gnngnngngg nngnngngg

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knngnnknkn nnknngnnkn nn

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<212> DNA

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 motif searched by protocol 2

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<220>

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<222> (11)..(13)

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      motif searched by protocol 2
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22

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<220>
<223> Description of Artificial Sequence:target site DNA
      motif searched by protocol 2
```

```
<220>
<221> modified_base
<222> (11)..(13)
<223> n = g, a, c or t, may be present or absent
```

23

```
<220>
<223> Description of Artificial Sequence:target site DNA
      motif searched by protocol 2
```

```
<220>
<221> modified_base
<222> (10)..(12)
<223> n = q, a, c or t, may be present or absent
```

22

```
<210> 41
<211> 23
<212> DNA
<213> Artificial Sequence
```

```
<220>
<223> Description of Artificial Sequence:target site DNA
      motif searched by protocol 2
```

```
<220>
<221> modified_base
<222> (1)..(23)
<223> n = g, a, c or t
```

```
<220>
<221> modified_base
<222> (11)..(13)
<223> n = g, a, c or t, may be present or absent
```

23

```
<210> 42
<211> 22
<212> DNA
<213> Artificial Sequence
```

```
<220>
<223> Description of Artificial Sequence:target site DNA
      motif searched by protocol 2
```

```
<220>  
<221> modified_base  
<222> (1)..(22)  
<223> n = g, a, c or t
```

```
<220>
<221> modified_base
<222> (10)..(12)
<223> n = g, a, c or t, may be present or absent
```

22

<210> 43
 <211> 23
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:target site DNA
 motif searched by protocol 2

<220>
 <221> modified_base
 <222> (1)..(23)
 <223> n = g, a, c or t

<220>
 <221> modified_base
 <222> (11)..(13)
 <223> n = g, a, c or t, may be present or absent

<400> 43
 knnkngggnnn nnnknggnnk nnn

23

<210> 44
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:target site DNA
 motif searched by protocol 2

<220>
 <221> modified_base
 <222> (1)..(22)
 <223> n = g, a, c or t

<220>
 <221> modified_base
 <222> (10)..(12)
 <223> n = g, a, c or t, may be present or absent

<400> 44
 knnkngggnnn nnknnknggn nn

22

<210> 45
 <211> 23
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:target site DNA
 motif searched by protocol 2

<220>
 <221> modified_base
 <222> (1)..(23)
 <223> n = g, a, c or t


```
<400> 45
knnknqgnnn nnnknnknqg nnn
```

```
<210> 46
<211> 22
<212> DNA
<213> Artificial Sequence
```

```
<220>
<223> Description of Artificial Sequence:target site DNA
      motif searched by protocol 2
```

```
<220>
<221> modified_base
<222> (1)..(22)
<223> n = q, a, c or t
```

```
<220>
<221> modified_base
<222> (10)..(12)
<223> n = q, a, c or t, may be present or absent
```

<400> 46
knnknqgnnn nnknnknknkn gg

22

```
<210> 47
<211> 23
<212> DNA
<213> Artificial Sequence
```

```
<220>
<223> Description of Artificial Sequence:target site DNA
      motif searched by protocol 2
```

```
<220>
<221> modified_base
<222> (1)..(23)
<223> n = g, a, c or t
```

```

<220>
<221> modified_base
<222> (11)..(13)
<223> n = g, a, c or t, may be present or absent

```

<400> 47
knnknggnnn nnnknnknnk ngg

23

```
<210> 48
<211> 22
<212> DNA
<213> Artificial Sequence
```

<220>
 <223> Description of Artificial Sequence:target site DNA
 motif searched by protocol 2

<220>
 <221> modified_base
 <222> (1)..(22)
 <223> n = g, a, c or t

<220>
 <221> modified_base
 <222> (11)..(12)
 <223> n = g, a, c or t, may be present or absent

<400> 48
 knnknnknngg nnknnggnkn nn

22

<210> 49
 <211> 23
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:target site DNA
 motif searched by protocol 2

<220>
 <221> modified_base
 <222> (1)..(23)
 <223> n = g, a, c or t

<220>
 <221> modified_base
 <222> (12)..(13)
 <223> n = g, a, c or t, may be present or absent

<400> 49
 knnknnknngg nnnknnggnkn nnn

23

<210> 50
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:target site DNA
 motif searched by protocol 2

<220>
 <221> modified_base
 <222> (1)..(22)
 <223> n = g, a, c or t

<220>
 <221> modified_base
 <222> (11)..(12)
 <223> n = g, a, c or t, may be present or absent

<400> 50
knnknnkngg nnknnknggn nn

22

<210> 51
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:target site DNA
motif searched by protocol 2

<220>
<221> modified_base
<222> (1)..(23)
<223> n = g, a, c or t

<220>
<221> modified_base
<222> (12)..(13)
<223> n = g, a, c or t, may be present or absent

<400> 51
knnknnkngg nnnknnkngg nnn

23

<210> 52
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:target site DNA
motif searched by protocol 2

<220>
<221> modified_base
<222> (1)..(22)
<223> n = g, a, c or t

<220>
<221> modified_base
<222> (11)..(12)
<223> n = g, a, c or t, may be present or absent

<400> 52
knnknnkngg nnknnknnkn gg

22

<210> 53
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:target site DNA
motif searched by protocol 2

<220>
 <221> modified_base
 <222> (1)..(23)
 <223> n = g, a, c or t

<220>
 <221> modified_base
 <222> (12)..(13)
 <223> n = g, a, c or t, may be present or absent

<400> 53
 knnknnknngg nnnknnknnk ngg

23

<210> 54
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:target site DNA
 motif searched by protocol 2

<220>
 <221> modified_base
 <222> (1)..(19)
 <223> n = g, a, c or t

<400> 54
 knnknnknngg nggnnknnn

19

<210> 55
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:target site DNA
 motif searched by protocol 2

<220>
 <221> modified_base
 <222> (1)..(19)
 <223> n = g, a, c or t

<400> 55
 knnknnknngg nnknngnnn

19

<210> 56
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:target site DNA
 motif searched by protocol 2

<220>
 <221> modified_base
 <222> (1)..(19)
 <223> n = g, a, c or t

<400> 56
 knnknknkngg nnknknkngg

19

<210> 57
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:target site DNA
 motif searched by protocol 3

<220>
 <221> modified_base
 <222> (1)..(22)
 <223> n = g, a, c or t

<220>
 <221> modified_base
 <222> (10)..(12)
 <223> n = g, a, c or t, may be present or absent

<400> 57
 kngknknknkn nnknknknkn nn

22

<210> 58
 <211> 23
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:target site DNA
 motif searched by protocol 3

<220>
 <221> modified_base
 <222> (1)..(23)
 <223> n = g, a, c or t

<220>
 <221> modified_base
 <222> (11)..(13)
 <223> n = g, a, c or t, may be present or absent

<400> 58
 kngknknknkn nnknknknkn nnn

23

<210> 59
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:target site DNA
 motif searched by protocol 3

<220>
 <221> modified_base
 <222> (1)..(22)
 <223> n = g, a, c or t

<220>
 <221> modified_base
 <222> (10)..(12)
 <223> n = g, a, c or t, may be present or absent

<400> 59
 kngknnknnn nnknnkngkn nn

22

<210> 60
 <211> 23
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:target site DNA
 motif searched by protocol 3

<220>
 <221> modified_base
 <222> (1)..(23)
 <223> n = g, a, c or t

<220>
 <221> modified_base
 <222> (11)..(13)
 <223> n = g, a, c or t, may be present or absent

<400> 60
 kngknnknnn nnnknnkngk nnn

23

<210> 61
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:target site DNA
 motif searched by protocol 3

<220>
 <221> modified_base
 <222> (1)..(22)
 <223> n = g, a, c or t

<220>
 <221> modified_base
 <222> (10)..(12)
 <223> n = g, a, c or t, may be present or absent

22

```
<220>
<223> Description of Artificial Sequence:target site DNA
motif searched by protocol 3
```

```
<220>
<221> modified_base
<222> (11)..(13)
<223> n = g, a, c or t, may be present or absent
```

23

```
<220>
<223> Description of Artificial Sequence:target site DNA
      motif searched by protocol 3
```

```

<220>
<221> modified_base
<222> (10)..(12)
<223> n = g, a, c or t, may be present or absent

```

22

```
<220>
<223> Description of Artificial Sequence:target site DNA
      motif searched by protocol 3
```

```
<220>
<221> modified_base
<222> (11)..(13)
<223> n = g, a, c or t, may be present or absent
```

23

```
<220>
<223> Description of Artificial Sequence:target site DNA
      motif searched by protocol 3
```

```

<220>
<221> modified_base
<222> (10)..(12)
<223> n = g, a, c or t, may be present or absent

```

22

<220>
<223> Description of Artificial Sequence:target site DNA
motif searched by protocol 3

```

<220>
<221> modified_base
<222> (11)..(13)
<223> n = g, a, c or t, may be present or absent

```

23

<210> 67
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:target site DNA
 motif searched by protocol 3

<220>

<221> modified_base

<222> (1)..(22)

<223> n = g, a, c or t

<220>

<221> modified_base

<222> (10)..(12)

<223> n = g, a, c or t, may be present or absent

<400> 67

knnkngknnn nnknnknnkn gk

22

<210> 68

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:target site DNA
 motif searched by protocol 3

<220>

<221> modified_base

<222> (1)..(23)

<223> n = g, a, c or t

<220>

<221> modified_base

<222> (11)..(13)

<223> n = g, a, c or t, may be present or absent

<400> 68

knnkngknnn nnnknnknnk ngk

23

<210> 69

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:target site DNA
 motif searched by protocol 3

<220>

<221> modified_base

<222> (1)..(22)

<223> n = g, a, c or t

<220>
 <221> modified_base
 <222> (11)..(12)
 <223> n = g, a, c or t, may be present or absent

<400> 69
 knnknknkngk nnknngknnkn nn

22

<210> 70
 <211> 23
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:target site DNA
 motif searched by protocol 3

<220>
 <221> modified_base
 <222> (1)..(23)
 <223> n = g, a, c or t

<220>
 <221> modified_base
 <222> (12)..(13)
 <223> n = g, a, c or t, may be present or absent

<400> 70
 knnknknkngk nnnknngknnk nnn

23

<210> 71
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:target site DNA
 motif searched by protocol 3

<220>
 <221> modified_base
 <222> (1)..(22)
 <223> n = g, a, c or t

<220>
 <221> modified_base
 <222> (11)..(12)
 <223> n = g, a, c or t, may be present or absent

<400> 71
 knnknknkngk nnknknkngkn nn

22

<210> 72
 <211> 23
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:target site DNA
 motif searched by protocol 3

<220>
 <221> modified_base
 <222> (1)..(23)
 <223> n = g, a, c or t

<220>
 <221> modified_base
 <222> (12)..(13)
 <223> n = g, a, c or t, may be present or absent

<400> 72
 knnknnkngk nnnknnkngk nnn

23

<210> 73
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:target site DNA
 motif searched by protocol 3

<220>
 <221> modified_base
 <222> (1)..(22)
 <223> n = g, a, c or t

<220>
 <221> modified_base
 <222> (11)..(12)
 <223> n = g, a, c or t, may be present or absent

<400> 73
 knnknnkngk nnknnknnkn gk

22

<210> 74
 <211> 23
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:target site DNA
 motif searched by protocol 3

<220>
 <221> modified_base
 <222> (1)..(23)
 <223> n = g, a, c or t

<220>
 <221> modified_base
 <222> (12)..(13)
 <223> n = g, a, c or t, may be present or absent

23

<220>
<223> Description of Artificial Sequence:target site DNA
motif searched by protocol 3

19

```
<220>
<223> Description of Artificial Sequence:target site DNA
      motif searched by protocol 3
```

19

<220>
<223> Description of Artificial Sequence:target site DNA
motif searched by protocol 3

19

<210> 78
 <211> 10
 <212> DNA
 <213> Glycine max

<220>
 <223> soybean FAD2-1 cDNA ZFP target segment FAD 1

<400> 78
 gaggttagagg 10

<210> 79
 <211> 10
 <212> DNA
 <213> Glycine max

<220>
 <223> soybean FAD2-1 cDNA target segment FAD 2

<400> 79
 gtcgtgtgga 10

<210> 80
 <211> 10
 <212> DNA
 <213> Glycine max

<220>
 <223> soybean FAD2-1 cDNA target segment FAD 3

<400> 80
 gttgaggaag 10

<210> 81
 <211> 10
 <212> DNA
 <213> Glycine max

<220>
 <223> soybean FAD2-1 cDNA target segment FAD 4

<400> 81
 gaggtggaag 10

<210> 82
 <211> 10
 <212> DNA
 <213> Glycine max

<220>
 <223> soybean FAD2-1 cDNA target segment FAD 5

<400> 82
 taggtggtga 10

<210> 83
 <211> 12
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:test sequence

<400> 83
 agtgcgcggt gc 12

<210> 84
 <211> 10
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:target site
 with base immediately to the 3' side of target
 site

<400> 84
 agtgcgcggt 10

<210> 85
 <211> 10
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:target site
 with base immediately to the 3' side of target
 site

<400> 85
 gtgcgcggtg 10

<210> 86
 <211> 10
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:target site
 with base immediately to the 3' side of target
 site

<400> 86
 tgcgcggtgc 10

<210> 87
 <211> 10
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:target site
 with base immediately to the 3' side of target
 site

<220>
 <221> modified_base
 <222> (10)
 <223> n = undefined

<400> 87
 gcgcgggtgcn

10

<210> 88
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:finger F3 for
 ordered output from optimal design target site

<400> 88
 Glu Arg Asp His Leu Arg Thr
 1 5

<210> 89
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:finger F2 for
 ordered output from optimal design target site

<400> 89
 Arg Ser Asp Glu Leu Gln Arg
 1 5

<210> 90
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:finger F1 for
 ordered output from optimal design target site

<400> 90
 Arg Lys Asp Ser Leu Val Arg
 1 5

<210> 91
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: finger for
disordered output from optimal design target site

<400> 91

Arg Ser Asp Glu Leu Thr Arg
1 5

<210> 92

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: finger for
disordered output from optimal design target site

<400> 92

Arg Ser Asp Glu Arg Lys Arg
1 5

<210> 93

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: three finger
ZFP design using F3, F2 and F1 fingers for ordered
output from optimal design target site

<400> 93

Arg Lys Asp Ser Leu Val Arg Arg Ser Asp Glu Leu Gln Arg Glu Arg
1 5 10 15

Asp His Leu Arg Thr
20

<210> 94

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: ZFP sequence
(F1, F2 and F3) from SBS design GR-223

<400> 94

Arg Ser Ala Asp Leu Thr Arg Arg Ser Asp His Leu Thr Arg Glu Arg
1 5 10 15

Asp His Leu Arg Thr
20

<210> 95
 <211> 21
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:ZFP sequence
 (F1, F2 and F3) from Zif 268

<400> 95

Arg Ser Asp Glu Leu Thr Arg Arg Ser Asp His Leu Thr Thr Arg Ser
 1 5 10 15

Asp Glu Arg Lys Arg
 20

<210> 96
 <211> 21
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:ZFP sequence
 (F1, F2, F3) from SP1

<400> 96

Lys Thr Ser His Leu Arg Ala Arg Ser Asp Glu Leu Gln Arg Arg Ser
 1 5 10 15

Asp His Leu Ser Lys
 20

<210> 97
 <211> 21
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:ZFP sequence
 (F1, F2, F3) from SBS design GL-8.3.1

<400> 97

Arg Lys Asp Ser Leu Val Arg Thr Ser Asp His Leu Ala Ser Arg Ser
 1 5 10 15

Asp Asn Leu Thr Arg
 20